

AN EXTENDITUNICATE ASCUS IN THE ASCOSTROMATIC GENUS *MELIOLINA*

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ABSTRACT - The ascus of the ascostromatic Hawaiian species, *Meliolina sydowiana* Stevens, develops a thickened wall before ascosporeogenesis which thins significantly during the formation of its ascospores. Ultrastructural examination reveals that a *Couche D* layer stretches during ascosporeogenesis and does not extend during ascospore ejaculation. This type of ascostromatic ascus is termed the extenditunicate ascus.

RÉSUMÉ - L'asque de l'espèce hawaïenne ascostromatique *Meliolina sydowiana* Stevens développe une paroi épaisse avant l'ascosporogénèse, qui s'amincit nettement pendant la formation des ascospores. L'étude ultrastructurale révèle qu'une *Couche D* s'étire durant l'ascosporogénèse et ne s'étend pas pendant l'expulsion des ascospores. Ce type d'asque ascostromatique est appelé "extenditunique".

KEY WORDS : Ascomycete, ascostroma, ascostromatic ascus, *Meliolina sydowiana*, Hawaii.

INTRODUCTION

Pseudoprototunicate, deliquescent, and explosive are terms used to describe the dehiscence of a thin-walled asci formed in the ascostroma. These asci have in common a supposed ascospore dispersal stratagem involving wall dissipation or disintegration.

The development of the ascus is accounted for in the literature in two ways. One interpretation implies that the ascus wall is developmentally a single layer formed during the preascosporogenic growth phase of the ascus initial. Thus, when the ascospores are mature, the ascus wall dissolves or quickly breaks apart to release the ascospores. Another interpretation suggests that the preascosporogenically thick-walled ascus becomes thinned during ascospore formation and implies a thinning through an alteration of the secondary wall layer structure.

The ascus of *Meliolina* is an example of a thick walled ascus generally described as becoming thin during ascosporeogenesis. Stevens (1918, 1925, 1927) and Stevens & Roldan (1935) described the ascus as evanescent in the original description of *M. paulinae* (Stevens) Stevens, *M. philippinensis*, *M. saurauiae* Stevens & Roldan, and *M. sydowiana*. The ascus of *M. arborescens* (Sydow & Sydow) Sydow & Sydow was described as *fugacibus* (Sydow & Sydow, 1913, 1914), as was the ascus of *M. chardonii* Toro (Toro & Chardon, 1934). Hansford (1946) wrote that the asci of *M. malaccensis* (Saccardo) Trotter were "rounded but not thickened at the apex". The ascus of *Meliola octospora* Cooke was described as "breikeuligen, dunnwandigen" by Höhnel (1909). A specimen determined as *Meliola octospora* Cooke by M.C. Cooke (= *Meliolina octospora* (Cooke) Höhnel) was said by Hansford (1946) to be "broadly rounded at the apex, with firm thick wall". The ascus of *M. novae-zealandiae* Hansford (Hansford, 1954) was found to be "rounded at the apex and when young thickened there". Pirozynski (1974) characterized the ascus of *Meliolina mollis* (Berkeley & Broome) Höhnel as "bitunicate, broadly clavate to ovoid, uniformly thick-walled when young, becoming saccate, ... thick-walled and often papillate at the apex, evanescent..." The ascus of *M. subramanianii* Hughes & Pirozynski (Hughes & Pirozynski, 1985) was called "relatively undifferentiated".

MATERIALS AND METHODS

The ascus used in this study is that of *Meliolina sydowiana* Stevens. A voucher specimen is curated in Herbarium LAM as # 300350A. The collection was made by Don R. Reynolds, on 23 May 1988, from the Wahi a Wai Bog, Kauai, Hawaii, USA. The fungus was growing on the surface of the leaves of the Ohia, *Meterosideros polymorpha* Gaudichaud.

Light microscope observations were made with a Zeiss dissection microscope and a Nikon compound microscope. Whole and squash mounts and handcut cross sections were made of the ascocarps. The hymenial layer was dissected from KOH pretreated ascocarps. Final mounts for observations were made in lactophenol. Cytochemical tests were made according to the protocol of Eriksson (1981) and Baral (1987).

Two day old fruitbodies were teased from leaf surfaces and fixed for 48 hours with a solution of 2% glutaraldehyde, 2.5% paraformaldehyde and 1% acrolein in a 0.1M cacodylate buffer containing 0.4% CaCl. The material was then rinsed in buffer, dehydrated in a graded series of ETOH, followed by 100% propylene oxide. Infiltration of the embedding material began with a 12-hour soak in 50:50 propylene oxide and epoxy resin. Embed 812 (Electron Microscopy Sciences, Fort Washington, Pennsylvania, USA) was selected as the embedding medium because of its low viscosity (4.1cp) and rapid flow (0.535ml/sec) (Mascorro & Kirby, 1986). Thin-sections were poststained with aqueous uranyl acetate and Reynold's lead citrate (Rey-

nolds, 1963). Observations were made with a JOEL 100CXII electron microscope at 80 KV.

RESULTS

Light Microscopy (Fig. 1)

The ascocarp begins as a cluster of cells arising from one or more hyphae that traverse the surface of the leaf. The hymenial system is formed in the basal area of a chamber formed as the ascocarp develops from an apical meristematic area. The 2 nuclei of the N+N ascus cell (Fig. 1A) fuse to form a dikaryon with a conspicuous ring-shaped nucleolus. The growth of the ascus initial is directed by the diploid nucleus surrounded by a highly granular cytoplasm (Fig. 1A). Asci are formed among paraphyses (Fig. 1B) originating in the same basal cell mass as the ascogenous system. The ascus wall at this stage is thinnest in the lower regions of the ascus and somewhat thicker in the apex. The ascus growth is arrested prior to meiosis. Additional wall material is formed in the inner surface of the wall formed during the initial growth phase so that the ascus wall is conspicuously thicker in the apical region than in the lower area. Meiosis is indicated by the appearance of ascospore initials in the cytoplasm. Cross-septa divide the ascospores into first 1 and then 3 cells; finally 2 small cells are formed at the spore apex. The spore walls become thickened and then imbued with a melanoid pigmentation. The fully pigmented cells continue to enlarge so that the ascospore cells are enlarged to give the appearance of a constricting septum. The ascus wall at this stage is thinly stretched and contoured around the cluster of 8 ascospores (Fig. 1B). The ascospores are ejaculated via an apical opening. The empty ascus shows an inner wall with a highly undulate surface (Fig. 1C).

Electron Microscopy (Fig. 2-6)

The primary wall appears in the young ascus as a darkly staining outer boundary (Fig. 2). Irregularly shaped vacuoles are formed in the boundary between the protoplast and the primary wall in the apical area to the ascus. The deposition of the secondary wall material is completed by the onset of ascosporogenesis (Fig. 3). The banded or accordion configuration of the darkly staining microfibrils embedded in a lighter staining wall matrix is that of the *Couche* D. The ascospore initials formed during the initial stages of ascosporogenesis are generally elongate, with convoluted outlines (Fig. 3, 4). The ascospore initials assume a more rigid ovoid shape as the outer and cross walls become more pronounced (Fig. 1B, 4, 5, 6). Both the primary wall and the *Couche* D become thinner with the maturation of the ascospores. The vertical bands at the apex of the *Couche* D become less apparent (Fig. 5, 6) accompanied by a diminished diameter. The fully shaped ascospores are surrounded by a perisporic sac which may interface with the inner-

most surface of *Couche D* (Fig. 6). The surface of *Couche D* is highly convoluted at the protoplasmic interface.

DISCUSSION

The formation of the ascocarp is representative of ascostromatic development.

The development of the ascus of *Meliolina sydowiana* has the *Couche D* structural attributes of the fissitunicate ascus (Reynolds, 1970, 1989) and the rostrate ascus (Reynolds, 1987) and its variant (Bellemère & Letrouit-Galinou, 1981; Honegger, 1978) asci. The function of *Couche D* during ascosporeogenesis and the role in ascospore ejaculation are different from these ascus types (Fig. 7).

The growth of the young ascus is guided by a diploid nucleus and is bounded by a primary wall; the secondary wall material is deposited on the inner surface of the primary wall when the ascus initial has reached a maximum height; the secondary wall has a *Couche D* profile; ascosporeogenesis is accompanied by modification of the secondary wall layers.

The ascus studied differs from other ascostromatic ascus types in the function of the secondary wall layer. There is no extension of the *Couche D* when the ascospores are ejaculated. The primary wall and the secondary wall with the *Couche D* configuration are the only apparent wall layers. As the contorted ascospore initials become rigid with the development of walls, the ascus shape is modified with the assistance of the *Couche D*. The secondary wall becomes highly modified as the ascospores mature. When the ascospores mature, the secondary wall material is thinly stretched as compared to its presence immediately prior to the initiation of ascosporeogenesis with meiosis.

This type of ascolocular ascus is distinctive and warrants recognition. Thus, it is named extenditunicate. The prefix, *extendo*, is a Latin term meaning to stretch out; the suffix, *tunica*, is the Latin term meaning sleeve or covering, already used for several other types of ascolocular asci. The criteria for the extenditunicate ascus are: (1) the formation of a primary wall during the growth of the ascus initial cell; (2) a preascosporeogenesis increase in the wall mass with the secondary deposition of a wall material characterized by a *Couche D* configuration; (3) the reorganization of the ascus wall during ascosporeogenesis involving the stretching of the primary and secondary portions; (4) a postascosporeogenesis diminished *Couche D*; (5) ejaculation of the ascospores from the ascus without an extension of *Couche D* or other portions of the ascus wall as a tube or rostrum.

The extenditunicate ascus is likely to be found in several ascostromatic families. The telltale clue in the descriptive literature is an ascus wall that is noted to become thickened at about the time of ascospore formation and

then thin again as the ascospores mature; there is usually no report of an extension of the wall at the time of ascospore dispersal as would be expected in the case of the bitunicate ascus *sensu lato* (Reynolds, 1989).

The formation of the wall thickening begins in the early stages of meiosis (Uecker, 1977) in the Zopfiaceae and is complete by Metaphase I in *Zopfia rhizophila* Rabenhorst. The ascus wall is very thin at the time of the completion of ascosporogenesis, and the ascospores are released by the dissolution of the ascus wall (Hawksworth & Booth, 1974; Uecker, 1977). The ascus of *Caryspora* (Jeffers, 1940) and of *Ulospora* (Hawksworth, 1979) were described as bitunicate and evanescent. The genus *Lepidopterella* (Shearer & Crane, 1980) was assigned by Eriksson & Hawksworth (1986) to the Zopfiaceae although Shearer & Crane (1980) clearly described the extrusion of an endoascus from its ectoascus.

The Piedraiaceae may have the extenditunicate ascus. The wall of the ascus in *Piedraia hortae* (Brumpt) Fonseca & Leao apparently dissolves to release the ascospores according to Ciferri et al. (1956) and Takashio & Vanbreuseghem (1971). The illustrations of the former indicate an isodiametric ascus wall, and those of the latter indicate a thin-walled ascus with a slightly thickened apex.

The ascus of *Trabutia* (Barr, 1987) was described as "... thick walled in youngest stages, but at maturity is thin walled, stretched and distorted by the mature ascospores. No fissitunicate breaking of ectotunica or stretching of endotunica has been observed; instead the asci eventually break down and ascospores are freed in the centrum. These are definitely not bitunicate asci. *Trabutia* possesses unitunicate asci and must be removed from the Loculoascomycetes".

The ascus of the Leptopeltidiaceae is probably extenditunicate. This ascus has been considered to be both unitunicate (Barr, 1979) and an unconfirmed bitunicate according to Eriksson's (1981) interpretation of a statement by Müller (1979). These observations were influenced by the finding of Holm & Holm (1977) of a thick-walled ascus in *Leptopeltis nebulosa* (Petraik) Holm & Holm in which they could not demonstrate bitunicate dehiscence.

Bellemère & Hafellner (1982) observed that the "banded pattern" of the inner layer of the ascus wall is not restricted to bitunicate asci.

Certain parts of the apical apparatus of unitunicate asci were said to have similarity to the banded pattern portions of the bitunicate endotunica by Parguey-Leduc & Janex-Favre (1984). Fibrillar bands characteristic of *Couche D* were found in the apical apparatus of sarcoscyphaceous species (Samuelson, 1975) and *Lasiobolus monascus* Kimbrough of the Pezizales (Kimbrough & Benny, 1978) which appeared as thick, irregular bulges found immediately under the operculum. A similar type of ascus was found in *Theobolus* (Kimbrough, 1981; Samuelson & Kimbrough, 1978) and in *Didymosphaeria arachidicola* (Chochrjakov) Alcorn (Wyk et al., 1987).

Reference has been made to a wall that remains thin at all times, such as in *Meliola* (Luttrell, 1989), becoming distended about the mature ascospores. This ascus should be examined for the involvement of a *Couche D* in its development.

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FIGURES

Fig. 1 - Ascical stages of *Meliolima sydowiana* as seen with light microscopy. A - 2 views of the preascosporogenic ascus: ascus initial with 2 supposed fusion nuclei (Double arrows) with minimum formation of wall; young ascus with fusion nucleus (single arrow) with primary wall (arrows); ascus on right contains ascospores with early stages of wall pigmentation; the ascus on the left has fully mature, darkly pigmented ascospores. Note the copious gelatinous paraphyses. C - The postejaculation ascus. The empty ascus is somewhat obpyriform in outline from an enlarged basal to middle section which narrows leading to the truncate apex through which the ascospores have been expelled in the direction of the arrow. The inner surface of the ascus, the contracted *Couche D*, is undulate.

Fig. 2 - *Meliolima sydowiana*, premeiotic ascus (TEM). Apical view; elongate vacuoles occur at the interface of the primary wall and the protoplast, particularly in the ascus tip.

Fig. 3 to 6 - *Meliolima sydowiana*, postmeiotic ascus (TEM).

Fig. 3 - Ascus in early stage of ascosporogenesis (tangential view); a darkly staining, non-structured primary wall overlays a secondary layer which has the banded pattern of the *Couche D*; the undivided ascospore initials have contorted outlines, and minimal wall formation; paraphyses can be seen in cross and longitudinal views at the periphery of the ascus. Fig. 4 - Cross-septa have begun to form in the still contorted but more polarized ascospore initials. Fig. 5 - The ascospore initials have less contortion as the septa become flatter; note the matched contours of the ascospore initial and the inner surface of the *Couche D* where they are in contact. The width of the *Couche D* is less than that seen in Fig. 4. Fig. 6 - The outer walls and septa of the mature ascospores are rigid; a perisporic sac is present; the primary wall is thinner than illustrated in earlier stages, the *Couche D* is minimally present and the characteristic banding pattern has been lost.

Fig. 7 - A diagrammatic representation of the stages of formation of the extenditunicate ascus; the ascus apex and the ultimate exit area for the ascospores is indicated by an arrow. A. The postmeiotic ascus has a fully formed primary wall and the initial deposition of the secondary wall has the banded pattern characteristic of *Couche D*. B. The postmeiotic ascus in the early stages of ascosporogenesis; the primary wall and the secondary wall with the banded pattern of the *Couche D* are fully formed; the highly contorted ascospore initials have been delineated from the protoplast. C. The mature, darkly pigmented ascospores surrounded by a perisporic sac are present in an ascus with the primary wall and *Couche D* thinned from stretching during ascosporogenesis. D. The ascus after the ejaculation of the ascospores; the inner surface of *Couche D* has an undulate pattern resulting from its contraction during the release of the ascus contents.

AS = ascospore; ASI = ascospore initial; CD = *Couche D*; P = paraphysis; Ps = perisporic sac; PW = primary wall; S = septum.

Fig. 1 - Phases ascales de *Meliolina sydowiana* en microscopie optique. A - 2 asques préascosporogènes : initiation d'asque avec 2 noyaux supposés en fusion (double flèche), peu de paroi; jeune asque avec noyau fusionné (flèche), paroi primaire. B - Asques postascosporogènes avec ascospores bien formées, la paroi de l'asque est fine (flèches); l'asque de droite contient des ascospores aux premiers stades de pigmentation de leurs parois; l'asque de gauche est mûr, les ascospores sont fortement pigmentées. Noter les nombreuses paraphyses gélatineuses. C - L'asque vide, après éjection, est obpyriforme, large à la base et s'amincissant pour former l'apex tronqué par lequel les ascospores ont été expulsées en direction de la flèche. La face interne de l'asque est ondulée (*Couche D* contractée).

Fig. 2 - *Meliolina sydowiana*, asque préméiotique (MET). Vue apicale; des vacuoles allongées apparaissent à l'interface de la paroi primaire et du protoplaste, particulièrement à la pointe de l'asque.

Fig. 3 à 6 - *Meliolina sydowiana*, asque postméiotique (MET).

Fig. 3 - Asque jeune; la paroi primaire non structurée recouvre une couche secondaire, typique de la *Couche D*; les jeunes ascospores non divisées ont des contours irréguliers et peu de paroi; des paraphyses apparaissent en coupe longitudinale à la périphérie de l'asque. Fig. 4 - Les cloisons transversales commencent à se former dans les jeunes ascospores encore irrégulières mais plus polarisées. Fig. 5 - Les jeunes ascospores sont plus régulières à mesure que les cloisons s'épaississent; noter la correspondance entre le contour de la jeune ascospore et celui de la face interne de la *Couche D*, lorsqu'elles sont en contact. L'épaisseur de la *Couche D* est inférieure à celle observée sur la fig. 4. Fig. 6 - Les parois externes et les cloisons des ascospores mûres sont rigides; présence d'un sac périsporique; la paroi primaire est plus fine que celle illustrée dans les stades précédents, la *Couche D* est peu importante et ses caractéristiques ont disparu.

Fig. 7 - Représentation schématique des stades de formation d'un asque "extendituniqué"; l'apex, point d'éjection des ascospores, est indiqué par une flèche. A - L'asque postméiotique a une paroi primaire bien formée et le début de la paroi secondaire a l'aspect strié caractéristique de la *Couche D*. B - Asque postméiotique dans les premiers stades de l'ascosporogénèse; la paroi primaire et la paroi secondaire striée, typique de la *Couche D*, sont bien formées; les jeunes ascospores très irrégulières sont délimitées du protoplaste. C. Les ascospores mûres, fortement pigmentées et entourées du sac périsporique, sont dans un asque ayant une paroi primaire et une *Couche D* amincie par rapport à celle observée durant l'ascosporogénèse. D - Asque après éjection des ascospores; la face interne de la *Couche D* est ondulée à la suite de sa contraction pour évacuer le contenu de l'asque.

AS = ascospore; ASI = jeune ascospore; CD = *Couche D*; P = paraphyses; Ps = sac périsporique; PW = paroi primaire; S = cloison.

FIGURE 1

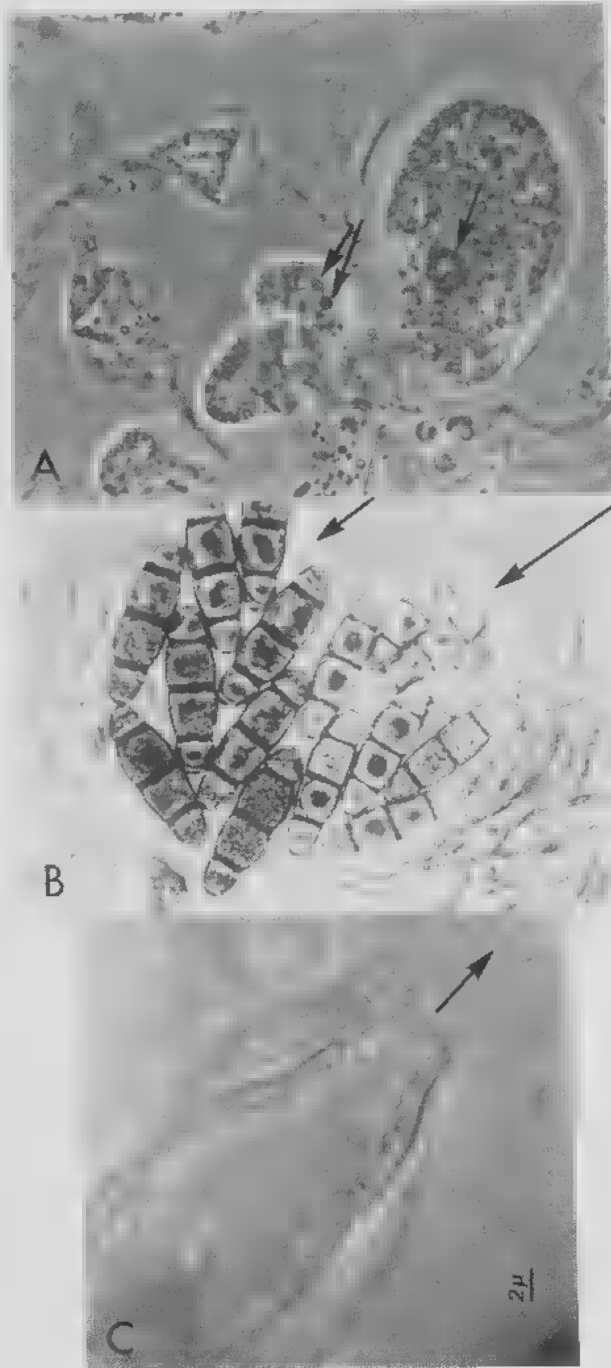


FIGURE 2

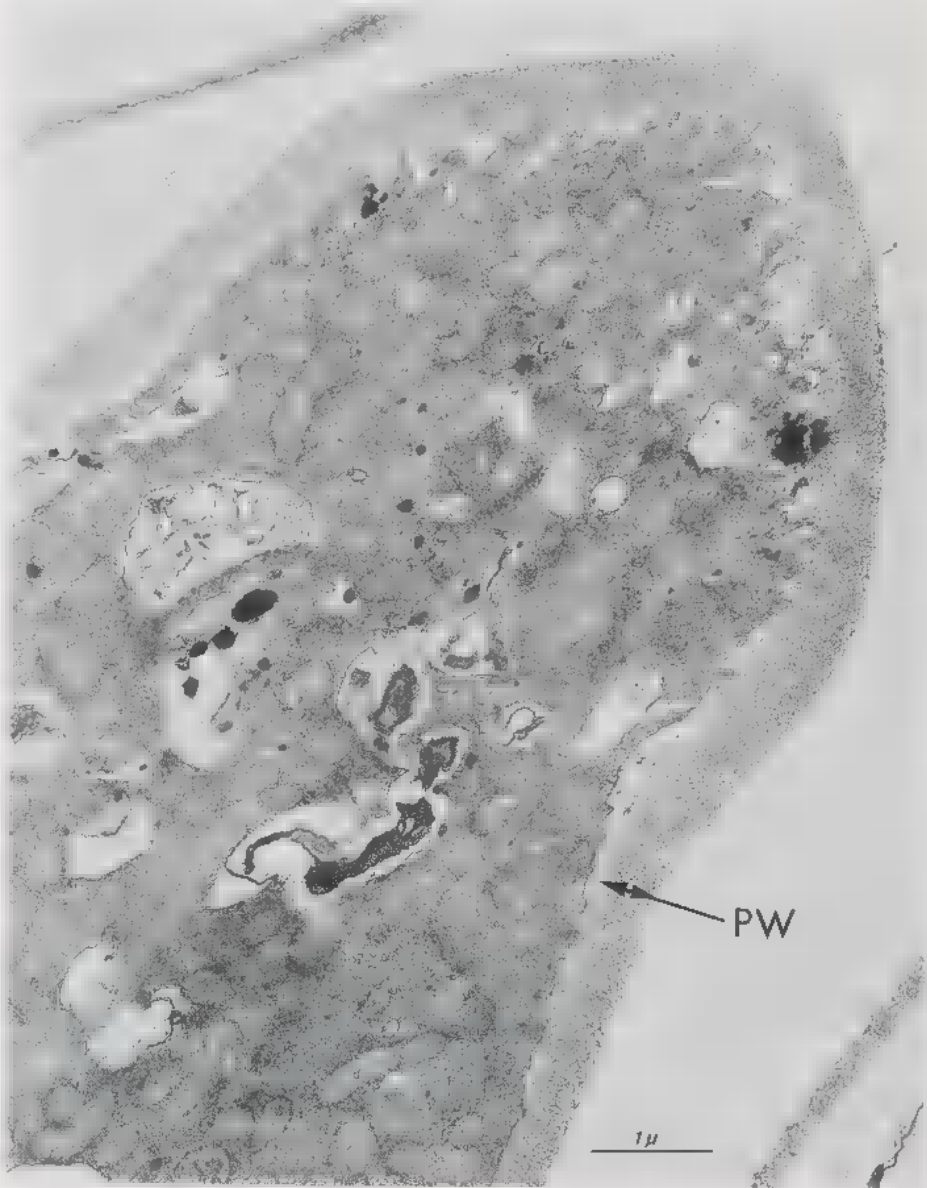


FIGURE 3

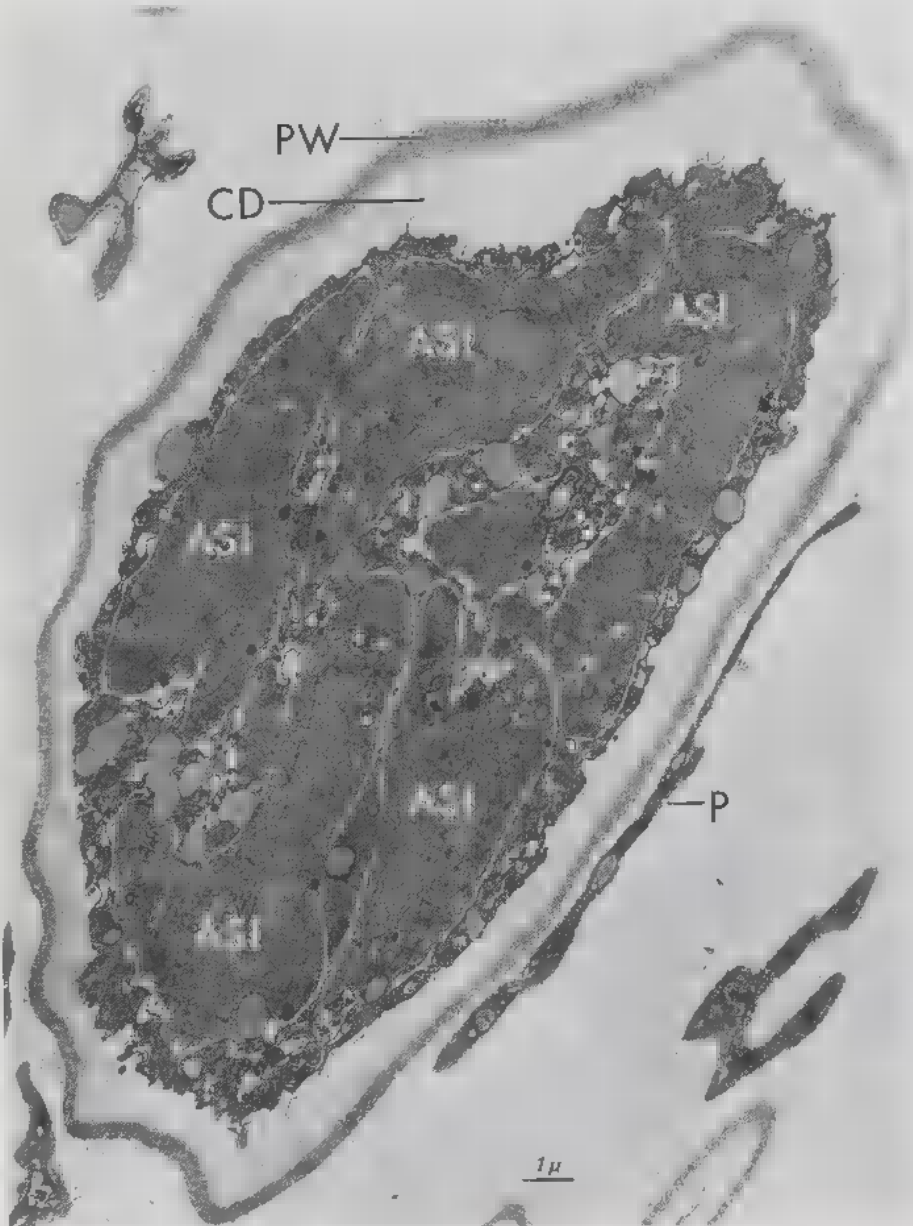


FIGURE 4





FIGURE 6

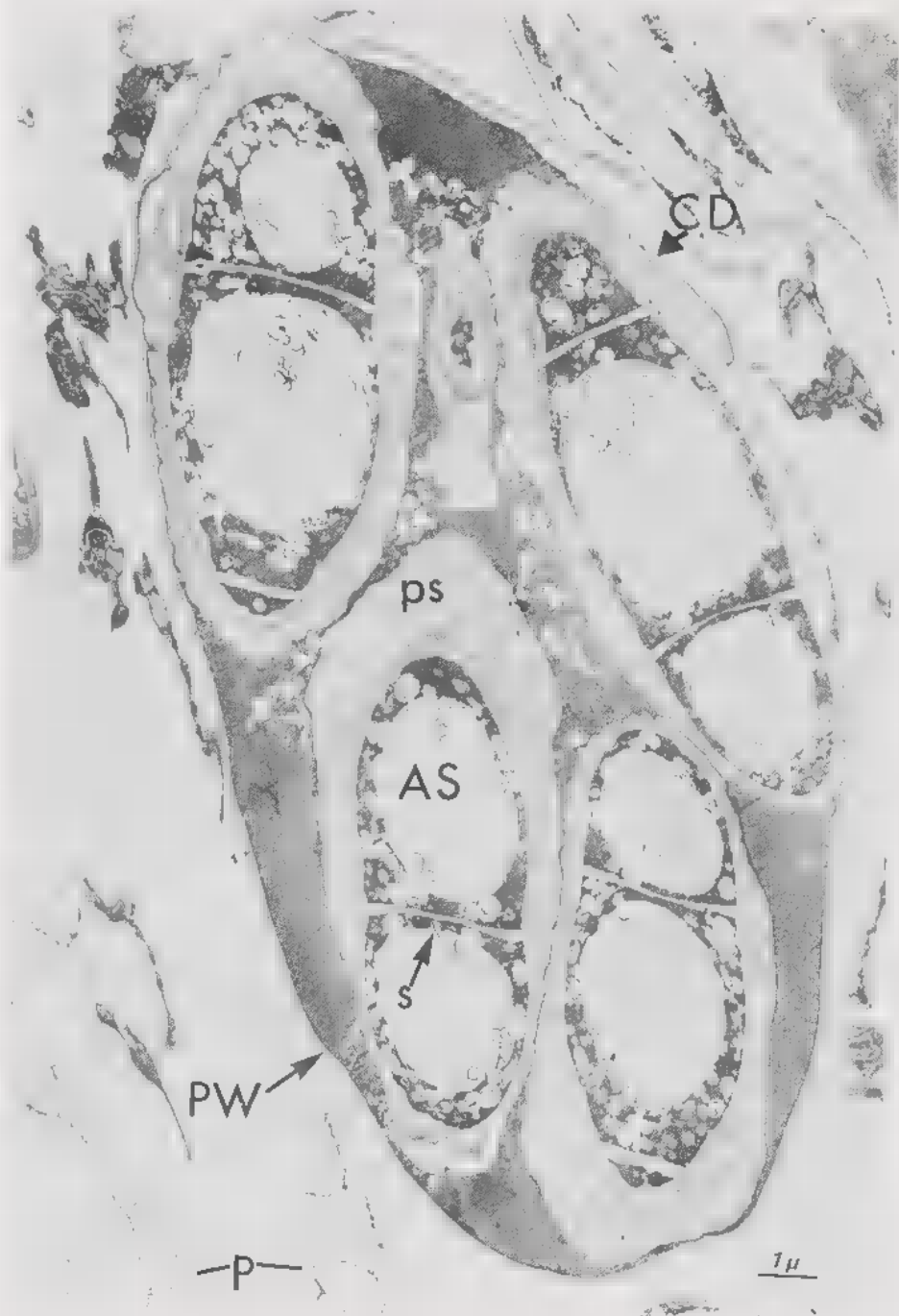


FIGURE 7

